UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/765,555	01/19/2001	Carlos F. Barbas III	278012001420	1190

25225 7590 05/21/2003

MORRISON & FOERSTER LLP
3811 VALLEY CENTRE DRIVE
SUITE 500
SAN DIEGO, CA 92130-2332

EXAMINER	
IBRAHIM, MEDINA AHMED	
ART UNIT	**PAPER NUMBER**
1638	

DATE MAILED: 05/21/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/765,555	BARBAS ET AL.	
	Examiner	Art Unit	
	Medina A Ibrahim	1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 March 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-138* is/are pending in the application.

4a) Of the above claim(s) *2,32-35,90,96 and 101-132* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-31,36-89,91-95,97-100 and 133-138* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *17* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Election/Restrictions

1. Applicant's election with traverse of Group II, claims 1, 3-31, 36-89, 91-95, 97-100 and 133-138) in Paper No. 16 is acknowledged. The traversal is on the ground(s) that claims of Groups II and IV can be examined together since the plant organelle of Group IV is a component of the plant/plant cells of Group II. Applicants request that the invention of Groups II and III be examined together as the nucleic acid of Groups II and IV can encode the ZFP of Group III. Applicant further requests that Group VIII be rejoined with Group II as the nucleic acids of Group VIII are the starting material and/or the products of the methods of Groups II-IV. Applicant asserts that there would be no extra search required for Group VIII if rejoined to Groups II-IV. Applicant further asserts that Group I can also be rejoined to Group III as they belong to the same class and subclass. The same argument applies to Groups VI and X, and Groups VII and XI. These arguments have been fully considered but not all are persuasive.

Applicant's argument regarding rejoining Groups IV and II is found persuasive, and therefore, Group IV is hereby rejoined with Group II. The arguments regarding coexamination of Group III or VIII with Group II are not persuasive for the following reasons: the invention of Group III requires an activator or a repressor protein linked to the zinc finger protein which is not required by the invention of Group II. Therefore, the two inventions have different starting material and a result have different modes of operation. In addition, the end products of the two groups will have different levels of effect. The invention of Group VIII is patentably distinct from the invention of Group of II

because it requires transformation of a cell for the production of zinc proteins, while the invention of Group II requires transformation and regeneration of plant to produce transgenic plants/plant cells. Therefore, Group III or VIII will not be rejoined with Group II, as it will create a search burden upon the Examiner. While the invention of Groups III and I are classified under same class/subclass, the two inventions use different starting material and, therefore, have different modes of operation. In addition, the literature search of Group I and III is divergent. While the zinc protein of Group VI and the fusion protein of Group X are classified under same class/subclass, the literature search of the groups is different because the invention of Group X requires fusion protein, the zinc finger protein of 2C7 fused to an effector domain of SID, that are not required by the invention of Group VI. In addition, there is no evidence in record that shows the inventions of Groups VI and Group X are obvious over each and therefore, are not patentably distinct. The same reason applies to Groups VII and XI. In addition, Applicant's assertion that if two groups are classified under same class/subclass they will not be searched as separate inventions and therefore would not be search burden upon the Examiner is incorrect because even if two inventions fall under same classification there is no reason to believe that their coexamination is not coextensive. The restriction requirement is still deemed proper and is therefore made FINAL.

Claims 1, 3-31, 36-89, 91-95, 97-100 and 133-138 are under examination.

Claims 1-138 are pending.

Claims 2, 32-35, 90, 96 and 101-132 are withdrawn from consideration as being drawn to a non-elected invention.

Priority

2. In the first line of the application, the status of the nonprovisional parent application no 09/620, 897, now converted to provisional no. 60/327, 552 should be updated. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application and priority applications by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

Drawings

1. The drawings filed on 01/19/2001 are approved by the Examiner.

Claim Objections

Claim 98 is objected to because a plant regenerated from a plant is unclear. It is suggested that ---cells--- is inserted after the second "plant".

In claim 57, "culturing is in planta" is unclear. It is suggested that the phrase be replaced with ----the plant cell is cultured in planta---.

Claim Rejections - 35 USC § 112

2. The following is a quotation of the **second** paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

3. Claims 1, 3-31, 36-69, 83-84 and 133-138 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 4 are indefinite in the recitation of "providing plant cells with a zinc finger protein" and "providing plant cells with an expression system for a zinc protein" as

it is unclear how the zinc finger protein and the expression system resulted in the plant. Dependent claims 5-31, 36-69, and claims 133-138.

In claim 3, it is unclear how the nucleotide sequence encoding the zinc finger protein resulted in the plant.

Claim 16 is indefinite in the recitation of a "small molecule" which is not defined in the specification. What is sought for protection is unclear. In addition, "small" is a relative term lacking comparative basis, and hence it is not clear what is encompassed by the claim.

Claim 25 is indefinite because the metes and bounds of a " taste molecule" is unclear.

Claim 26 is indefinite in the recitation of " bad taste molecule" as the metes and bounds of the claim is unclear. In addition, "bad" is a relative term lacking comparative basis, and hence it is unclear what is encompassed.

In claim 27, a " metabolic pathway that is heterologous to a plant" is not defined in the specification, and it is unclear how a whole metabolic pathway involved by numerous genes can be inserted into a plant cell. The metes and bounds of the claim is unclear.

In claim 28, the recitation of "metabolic pathway enhances an input or output trait in a plant or seed" is unclear, and the specification fails to define what is encompassed by the phrase. The claim is open to a variety of interpretations.

In claim 49 is indefinite because is unclear how plant cells can constitute all the cells of an intact plant.

Claims 83 and 84 are indefinite because claim 76 does not recite a first promoter.

In claims 133-134, 136, and 138, "derived" renders the claims indefinite because it is unclear what is being retained in the derived product.

Claim Rejections - 35 USC § 112

4. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claims 1, 3-30, 36-89, 91-95, 97-100 and 133-138 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of modulating the expression level of the APETALA3 (AP3) gene in Arabidopsis, and the gene encoding *myo* inositol 1-phosphate synthase (MIPS) in maize by transforming the plant cells with specific nucleotide sequences encoding zinc finger proteins of ZFPm1 (SEQ ID NO: 38), ZFPm2 (SEQ ID NO: 39), ZFPm3 (SEQ ID NO: 40), and ZFPm4 (SEQ ID NO: 41) that are designed for the MIPS gene, and the ZFPAP3 (SEQ ID NO:43) for AP3 gene in Arabidopsis, an expression vector comprising said specific nucleotide sequences, transformed plant, plant cells, and seed comprising said expression vector, does not reasonably provide enablement for any method to modulate the expression of any target gene with any zinc finger protein capable of binding to said target gene in plant cells, or any method of providing plant cells with any zinc finger protein, a method that modulating expression of a target gene encoding a protein that controls a metabolic pathway in a plant cell or a method that employs a framework derived from a zinc finger protein that is known in the prior art as

of the filing date of this application. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The claims are broadly drawn to any method for modulating gene expression in plant cells comprising providing plant cells with a zinc finger protein that binds to a target nucleotide sequence within the target gene or a complementary strand thereof, wherein the modulation is either repression or activation, an expression vector comprising a nucleotide sequence encoding any zinc finger protein and genetically modified plant, plant cells and seed comprising said expression vector. The claims also encompass target genes encoding products that affect biosynthesis, modification, cellular trafficking, metabolism and degradation of a protein or a peptide including enzymes, transport proteins such as ion channels and pump nutrients, nutrient and storage proteins, contractile or motile proteins, structural proteins, defense proteins, proteins that control metabolic pathways, regulatory proteins such as antibodies, an oligonucleotide, nucleic acids including RNA, DNA and DNP, a vitamin, an oligosaccharide, a taste molecule, a carbohydrate, a lipid, antioxidant, sugar, flavanoid and a small molecule. The claims further encompass target nucleotide sequence that can be exogenous or endogenous to the target gene and can be in any place in the target gene, and a zinc finger protein comprising multiple finger regions of from 2 to 10 amino acid residues and linker regions. The claims also encompass finger proteins directed to a specific organelles of a plant via transit peptides.

Applicant provides guidance for the construction of five zinc finger proteins ZFPm1 (SEQ ID NO: 38), ZFPm2 (SEQ ID NO: 39), ZFPm3 (SEQ ID NO: 40), and ZFPm4 (SEQ ID NO:41) that are specific for the MIPS gene, and the ZFPAP3 (SEQ ID NO:42) that is designed to bind AP3 gene in Arabidopsis. The human zinc finger protein Sp1C has been used to serve as the framework for construction of said new ZFPs. Applicant also provides guidance for a method for controlling expression of the reporter gene luciferase in tobacco and maize cells, the APETALA3 (AP3) gene in Arabidopsis, and the gene encoding *myo* inositol 1-phosphate synthase (MIPS) in maize by transforming the plant/plant cell with a construct comprising a nucleotide sequence encoding ZFPm1, ZFPm2, ZFPm3, ZFPm4, and the ZFPAP3. In one experiment, Applicant teaches transient regulation of the expression of reporter gene luciferase in plant cells, via the zinc finger protein, 2C7 (Figure 1). In another experiment, Applicant has shown the position effect of zinc finger protein binding site on the activation of the luciferase construct in tobacco cells by using six tandem repeats of 2C7 binding sites (6X2C7), (Example 2; Figure 2). Applicant further teaches a method for selection of suitable zinc finger protein binding sites for endogenous MIPS and AP3 genes by using GNN and TGA triplet recognition codes (Example 3; Table 4) and has shown that the activity of zinc finger proteins with 6 and 3-fingers can modulate the expression of the target gene AP3 (Examples 3 and 10).

Applicant has not provided sufficient guidance for a method wherein any gene other than the MIPS gene and the AP3 of Arabidopsis is regulated through the expression of a zinc finger protein. Applicant has not shown that any other genes

including genes encoding proteins listed in claims 16, 23, 25-26 and 70 could be regulated by this method. Although Applicant has successfully designed a panel of ZFPs that bind to diverse DNA sequences and that are capable of regulating expression of endogenous AP3 and MIPs genes, it is not clear whether a suitable zinc finger protein "specifically binding" to a target site in a huge genome like the plant genome can be designed for the modification of any target gene without undue experimentation, since the mechanisms of gene regulation and zinc finger protein-DNA binding are complex phenomena and yet to understand.

Beerli et al. (PNAS USA 97, 1495-1500, 2000) teach unpredictability relating to regulating an endogenous cellular gene with designed zinc finger proteins. Beerli teaches that the transcriptional finger proteins E3-KRAB and E3-VP64 didn't affect the expression of target genes ErbB-1 and ErbB-2. Beerli teaches that not all genes would have a suitable 5' UTR, and not all zinc finger proteins bind their respective target sequence with high specificity and not all zinc finger proteins are able to discriminate between their cognate and very closely related DNA sequences. Beerli further teaches that non-specific binding of related DNA sequences by designed finger proteins may lead to significant side effects (see page 1498, first paragraph and the section under "*Requirements for Imposing Specific Regulation on Endogenous Genes*).

Further, while numerous studies have been conducted on the regulation of endogenous genes by designed zinc finger proteins in animal cells, the use of artificial zinc finger chimeras to regulate expression of endogenous genes in transgenic plants for a desired agronomic trait is at an early age.

Therefore, given the breadth of the claims, the complexity of the invention, i.e., upregulation and downregulation of endogenous cellular genes via controlled zinc finger proteins, lack of sufficient guidance in the specification regarding the regulation of any gene including those listed in the claims, unpredictability inherent with respect to regulating endogenous cellular genes with designed zinc finger proteins as evidenced by Beerli et al above, and the state of the art regarding gene regulation with designed zinc finger proteins in transgenic plants, the claimed invention is not enabled throughout the broad scope.

See *Amgen Inc. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ 2d 1016 at 1027 (Fed. Cir. 1991) where it is taught that the disclosure of a few gene sequences did not enable claims broadly drawn to any analog thereof.

Claims 31 and 76-89, 91-95, and 97-98 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The claims are directed to a method to modulate the expression of a target gene in plant cells by expressing a zinc finger protein that specifically binds to target nucleotide sequence within the target gene, wherein the method is used for treating a disorder associated with abnormal expression of the target, and a transgenic plant, plant cells and seed expressing a zinc finger protein that specifically binds to a target

nucleotide sequence within a target gene, to modulate the expression of the target gene.

Applicant has not provided guidance for any method for treating abnormal expression of a target gene in plant cells by using a zinc finger protein or any other compound. The prior art does amend the deficiency. One skilled in the art who is willing to practice the invention is left with undue trial and error experimentation because neither the instant specification nor the prior art provides any guidance for how to identify a disorder associated with upnormal gene expression in plant cells and/or how to treat said abnormality with zinc finger proteins. Also, the claimed transgenic plant, plant cell and seed are not supported by an enabling disclosure because Applicant has not taught the agronomic benefit or how to use a transgenic plant cells/plant and seed with modulated gene expression. Therefore, given the lack of guidance, the nature of the invention, the state of the art, unpredictability inherent with respect to regulating endogenous cellular genes with designed zinc finger proteins as evidenced by Beerli et al (as discussed in the scope of enablement rejection above), the claimed invention is not enabled.

Written Description

Claims 1, 3-31, 36-89, 91-95, 97-100 and 133-138 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed

invention. The claimed invention does not meet the current written description requirement for the following reasons.

The claims are broadly drawn to any method for modulating gene expression in plant cells comprising providing plant cells with a zinc finger protein that specifically binds to a target nucleotide sequence within the target gene or a complementary strand thereof, wherein the modulation is either repression or activation, an expression vector comprising a nucleotide sequence encoding any zinc finger protein and genetically modified plant, plant cells and seed comprising said expression vector. The claims also encompass target genes encoding products that affect biosynthesis, modification, cellular trafficking, metabolism and degradation of a protein or a peptide including enzymes, transport proteins such as ion channels and pump nutrients, nutrient and storage proteins, contractile or motile proteins, structural proteins, defense proteins, proteins that control metabolic pathways, regulatory proteins such as antibodies, an oligonucleotide, nucleic acids including RNA, DNA and DNP, a vitamin, an oligosaccharide, a taste molecule, a carbohydrate, a lipid, antioxidant, sugar, flavanoid and a small molecule.

Applicant has not described a method that employs any and all zinc finger proteins, DNAs encoding them, and any and all target genes in a plant. It is unclear if the structures and functional domains of target genes of claims 16, 25-26, 29-31 are known in the art. Applicant has not described suitable binding sites in the 5'UTR, coding or 3' UTR of a multitude of target genes, and it is unclear how one can regulate the expression of a gene that is not described and/or whose cognate transcriptional factor

(natural) is not known. The specification describes a method that employs zinc finger proteins of ZFPm1 (SEQ ID NO: 38), ZFPm2 (SEQ ID NO: 39), ZFPm3 (SEQ ID NO: 40), and ZFPm4 (SEQ ID NO: 41) that are specific for the MIPS gene, and the ZFPAP3 (SEQ ID NO: 43) for AP3. These are genus claims. Since Applicant has not described zinc finger proteins that can specifically target any and all genes as broadly claimed, a method of controlling the expression of said target genes, expression vector, and transformed plant/plant cell/seed comprising said genes are similarly not described. Therefore, given the lack of description of representative species encompassed by the genus of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that Applicants were in possession of any and all zinc finger proteins capable of regulating expression of any endogenous target gene in a plant. See MPEP 2163.

See also *University of California v. Eli Lilly and Co.* 43 USPQ2d 1398 (Fed. Cir. 1997), where the court stated that to adequately describe a claimed genus, Applicant must describe a representative number of the species of the claimed genus, and that one of skill in the art should be able to "visualize or recognize the identity of members of the genus". See, also Written description Examination Guidelines published in Federal Registry/Vol. 66, No.4/Friday, January 5, 2001/Notices).

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 3-9, 13-17, 19-20, 22-25, 28-30, 38-39, 44, 47-48, 50-56, 58-60, 67-70, 73-78, 83-84, 88- 89, 91-93, 133, and 136-138 are rejected under 35 U.S.C. 102(b) as being anticipated by Yanagisawa et al (The Plant Cell, vol. 10, pp. 75-89, 1998).

The claims are drawn to a method of modulating the expression of a target gene encoding specified proteins or a product in plant cells comprising transforming plant cells with an expression vector comprising a nucleotide sequence encoding a zinc finger protein and a tissue-specific or an inducible promoter, said zinc finger protein specifically binds to a target nucleotide sequence within the target gene, wherein the target nucleotide which is either exogenous or endogenous to the target sequence is upstream, downstream, or within the coding region of the target region, wherein the expression of the zinc finger protein is controlled by a tissue-specific or an inducible promoter, and wherein the expression of said target gene in said plant cells is modulated. The claims are also drawn to a genetically modified plant cells comprising said zinc finger protein.

Yanagisawa et al teach a method of controlling the expression of a gene in maize leaf tissues by using maize Dof1 zinc finger protein; the method comprises co-transforming maize plant cells with expression constructs comprising either, Dof cDNA and hsp promoter, or hsp promoter and Dof1 added to the activation domain GALA4, or the constitutive 35SC4PPDC (phosphoenolpyruvate carboxylase) gene promoter operably linked to Dof1, and a reporter plasmid construct comprising CAT and truncated 35 S with or without synthetic Dof1 binding site (see Page 77, and Figures 2-3; page 81,

Figure 7). Results indicated that Dof1 binds specifically to the phosphoenolpyruvate carboxylase gene promoter in vitro and in vivo, and showed that Dof1 alone was sufficient to activate transcription by 5 fold (Figure 6; page 81). The addition of GALA in the transformation vector further increased the transcription by at least 1.5 fold. The results further showed that transcriptional activation by Dof1 was due to its selective binding to the target sequence (Figures 9 and 10). The cited reference teaches the structural and functional domains of Dof1 and Dof2 proteins, and a method of identifying their binding sites in the C4PEPC gene promoter (paragraph bridging pages 81 and 82). Yanagisawa et al have shown that Dof2 acts as a transcriptional repressor in plants, and that it can interact with Dof1 binding sites but lacks the activity for transcriptional activation (paragraph bridging pages 83 and 84, and Figure 11). These results indicate that the expression of a phosphoenolpyruvate carboxylase gene, a photosynthesis pathway gene, can be regulated with the zinc finger protein Dof. A change of phenotype is an inherent property of transgenic plant/plant cell having modulated target protein. Given the broad interpretation of claim 28, an increase of C4PEPC gene expression will inherently enhance photosynthesis of the plant, an output trait. Therefore, the cited reference teaches all claim limitations.

6. Claims 1, 3-7, 9, 17, 19-20, 22, 29-30, 39-41, 44, 47-48, 51-52, 54-56, 67-69, 74-78, 83, 85-86, 89, 91, 93, 133-138 are rejected under 35 U.S.C. 102(b) as being anticipated by DE Pater Sylvia et al (nucleic Acids Research, vol. 24 (23), pp. 4624-4631, 1996)

DE Pater Sylvia et al teach a method of activating gene expression in plant cells with ZAP1, zinc finger protein from Arabidopsis with transcriptional enhancing activity; the method comprising introducing an expression construct comprising ZAP1 cDNA under the control of a 35S promoter into Catharanthus roseus cells together with a reporter construct comprising gus under the control of a truncated 35S promoter fused to synthetic ZAP1 binding sites (page 4626, Materials and Methods; Figure 5). Results show that the expression of ZAP1 protein in the plant cells increased transcription level of GUS by 6 fold (Figure 10B and C). Results further showed that ZAP1 functions as a sequence-specific zinc finger protein type of transcriptional activator in plant cells (page 4628). The results further showed the sequence CGTTGACCGAG as the optimal binding site for ZAP1, which implies that ZAP1 can impose transcriptional activation an all target genes with said optimal sequence in the promoter region, such genes are barley alpha amylase and maize pathogen related PRms genes (page 4632, column 1). Therefore, DE Pater Sylvia discloses all claim limitations.

Claim Rejections - 35 USC § 103

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein

were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 3-17, 19-20, 21-25, 28-30, 31, 36, 38-45, 47-48, 50-56, 58-60, 67-70, 73-78, 80-89, 91-95, 97-99 and 133-138 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yanagisawa et al (The Plant Cell, vol. 10, pp. 75-89, 1998, Applicant's IDS), in view BARBAS et al (WO 9854311, Applicant's IDS).

Claims are drawn to a method for modulating gene expression in plant cells comprising transforming plant cells with an expression vector comprising a nucleotide sequence encoding a zinc finger protein that specifically binds to a target nucleotide sequence of a target gene or a complementary strand thereof, wherein the target nucleotide sequence comprises 18 nucleotides and is the formula (GNN) n, where n is an integer from 1 to 6, and N is A, T, C, G, and wherein modulation is either repression or activation. The claims also encompass target genes encoding products that affect biosynthesis, modification, cellular trafficking, metabolism and degradation of a protein or a peptide including enzymes, transport proteins such as ion channels and pump nutrients, nutrient and storage proteins, contractile or motile proteins, structural proteins, defense proteins, proteins that control metabolic pathways, regulatory proteins such as antibodies, an oligonucleotide, nucleic acids including RNA, DNA and DNP, a vitamin, an oligosaccharide, a taste molecule, a carbohydrate, a lipid, antioxidant, sugar,

flavanoid and a small molecule, and zinc finger proteins including natural or synthetic known in the art as of the filing date of this application, comprising plurality of zinc fingers, and with or without effector domain. Genetically modified plant, plant cells and seed are also claimed.

The teaching of Yanagisawa et al is discussed supra.

Yanagisawa et al do not teach a method that employs non-natural zinc finger proteins to control gene expression in plant cells, plant and seeds and the specific target nucleotide sequences of the claimed invention.

BARBAS disclose a method of regulating expression of genes with designed zinc finger proteins derived from wild type finger proteins such as TFIIIA and zif268 known in the art, and having specific recognition sites. BARBAS teaches a method of designing zinc finger proteins comprising at least 2 or 3 zinc fingers separated by linkers, and capable of binding to a cellular nucleotide sequence in a target gene to modulate (up or down) the transcriptional activity of the gene (Examples 1-14). The cited reference teaches that the target nucleotide sequence is the triplet codon formula NNS or NNK (and its complement NNM), wherein N is A, C, G, or T comprising at least 9, 14 and 16 base pairs (pages 12, 21 and 30). The cited reference further teaches plant expression vectors comprising a nucleotide sequence encoding finger protein operably linked to CaMV (constitutive) or hsp (inducible) promoter (page 38, first full paragraph) and suggests that the designed zinc finger proteins can be used to modulate expression of a target gene in plant cells (claim 18) to produce transgenic plants with a desired phenotype such as disease resistant transgenic plants by using methods known in the

art (page 48). In paragraph bridging pages 41 and 42, BARBAS teaches that zinc finger proteins can be used to treat cell-proliferative disorder, which may be of cellular or viral origin, in a plant. BARBAS further suggest that any of the wild type zinc finger proteins known in the art can be used with his method to design novel zinc finger proteins to bind any chosen DNA sequence (paragraph bridging pages 2 and 3; page 16). Given the broad applicability of the method as taught by BARBAS, the expression of a target gene encoding a heterologous antibody in a plant or plant cell is expected to be affected by a suitable zinc finger protein.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to utilize the method of controlling gene expression in plant cells with zinc finger protein as taught by Yanagisawa et al, and to modify that method by incorporating either the designed zinc finger proteins taught by BARBAS, or those obtainable from the wild type zinc finger proteins taught by Yanagisawa et al, by using the zinc finger protein design and selection methods taught by BARBAS. One of ordinary skill in the art would have been motivated to use design zinc finger proteins instead of wild type because BARBAS teaches the advantage of using designed zinc finger proteins (higher degree of specificity sufficient for being selective even within a genome) in enhancing or repressing expression of endogenous cellular genes. One would have had a reasonable expectation of success in carrying out the claimed invention as taught by Yanagisawa et al. In addition, methods of stably transforming plants with heterologous genes are known in the art as suggested by BARBAS.

Therefore, the invention as a whole was *prima facie* obvious at the time the invention was made.

Claims 1, 3-9, 17, 19-20, 21-24, 29-30, 31, 36, 38-45, 47-48, 51-52, 54-56, 58-60, 67-70, 73-78, 80-89, 91-94, 97-99 and 133-138 are rejected under 35 U.S.C. 103(a) as being unpatentable over DE Pater Sylvia (al (nucleic Acids Research, vol. 24 (23), pp. 4624-4631, 1996, Applicant's IDS) in view BARBAS et al (WO 9854311, Applicant's IDS).

The teachings of DE Pater Sylvia have been discussed *supra.*

DE Pater Sylvia et al do not teach a method that employs non-natural zinc finger proteins to control gene expression in plant cells, plant and seeds and the specific target nucleotide sequences of the claimed invention.

BARBAS disclose a method of regulating expression of genes with designed zinc finger proteins derived from wild type finger proteins such as TFIIIA and zif268 known in the art, and having specific recognition sites. BARBAS teaches a method of designing zinc finger proteins comprising at least 2 or 3 zinc fingers separated by linkers, and capable of binding to a cellular nucleotide sequence in a target gene to modulate (up or down) the transcriptional activity of the gene (Examples 1-14). The cited reference teaches that the target nucleotide sequence is the triplet codon formula NNS or NNK (and its complement NNM), wherein N is A, C, G, or T comprising at least 9, 14 and 16 base pairs (pages 12, 21 and 30). The cited reference further teaches plant expression vectors comprising a nucleotide sequence encoding finger protein operably linked to

CaMV (constitutive) or hsp (inducible) promoter (page 38, first full paragraph) and suggests that the designed zinc finger proteins can be used to modulate expression of a target gene in plant cells (claim 18) to produce transgenic plants with a desired phenotype such as disease resistant transgenic plants by using methods known in the art (page 48). In paragraph bridging pages 41 and 42, BARBAS teaches that zinc finger proteins can be used to treat cell-proliferative disorder, which may be of cellular or viral origin, in a plant. BARBAS further suggest that any of the wild type zinc finger proteins known in the art can be used with his method to design novel zinc finger proteins to bind any chosen DNA sequence (paragraph bridging pages 2 and 3; page 16). Given the broad applicability of the method as taught by BARBAS, the expression of a target gene encoding a heterologous antibody in a plant or plant cell is expected to be affected by a suitable zinc finger protein.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to utilize the method of controlling gene expression in plant cells with zinc finger protein as taught by DE Pater Sylvia, and to modify that method by incorporating either the designed zinc finger proteins taught by BARBAS, or those obtainable from the wild type zinc finger proteins taught by DE Pater Sylvia, by using the zinc finger protein design and selection methods taught by BARBAS. One of ordinary skill in the art would have been motivated to use design zinc finger proteins instead of wild type because BARBAS teaches the advantage of using designed zinc finger proteins (higher degree of specificity sufficient for being selective even within a genome) in enhancing or repressing expression of endogenous cellular genes. One

would have had a reasonable expectation of success in carrying out the claimed invention as taught by DE Pater Sylvia. In addition, methods of stably transforming plants with heterologous genes are known in the art as suggested by BARBAS.

Therefore, the invention as a whole was *prima facie* obvious at the time the invention was made.

Claims 1, 3-17, 19-20, 21-25, 28-30, 31, 36, 38-45, 47-48, 50-70, 73-78, 80-89, 91-95, 97-99 and 133-138 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yanagisawa et al (The Plant Cell, vol. 10, pp. 75-89, 1998, Applicant's IDS), in view BARBAS et al (WO 9854311, Applicant's IDS) as applied to claims 1, 3-17, 19-20, 21-25, 28-30, 31, 36-45, 47-48, 50-56, 58-60, 67-70, 73-78, 80-89, 91-95, 97-99 and 133-138 above, and further in view of Applicants' admitted prior art.

The teachings of Yanagisawa et al in view of BARBAS have been discussed *supra*.

Yanagisawa et al in view of BARBAS do not teach the inclusion of a transit peptide in the plant expression vector. However, the inclusion of a transit peptide that targets a desired protein to specific organelle of a plant in a plant transformation vector was well known in the prior art, as evidenced by Applicant (page 8, lines 11-20)).

Applicant's admitted prior art indicates that chloroplast, mitochondria and nucleus transit peptides were well known and widely used at the time Applicant's invention was filed.

Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to utilize any of the known transit peptide

sequences of the prior art, including the claimed chloroplast, mitochondria and nucleus transit peptides for their availability, in the transformation vector without any unexpected results. One skilled in the art would have been motivated to generate the claimed invention with a reasonable expectation of success.

Claims 1, 3-9, 17, 19-20, 21-24, 29-30, 36, 38-45, 47-48, 51-52, 54-56, 58-60, 61-70, 73-78, 80-89, 91-94, 97-99 and 133-138 are rejected under 35 U.S.C. 103(a) as being unpatentable over DE Pater Sylvia (al (nucleic Acids Research, vol. 24 (23), pp. 4624-4631, 1996, Applicant's IDS) in view BARBAS et al (WO 9854311, Applicant's IDS) as applied to claims 1, 3-9, 17, 19-20, 21-24, 27, 29-30, 36-45, 47-48, 51-52, 54-60, 67-70, 73-78, 80-89, 91-94, 97-99 and 133-138 above, and further in view of Applicants' admitted prior art.

The teachings of DE Pater Sylvia in view of BARBAS have been discussed *supra.*

DE Pater Sylvia et al in view of BARBAS do not teach the inclusion of a transit peptide in the plant expression vector. However, the inclusion of a transit peptide that targets a desired protein to specific organelle of a plant in a plant transformation vector was well known in the prior art, as evidenced by Applicant (page 8, lines 11-20)).

Applicant's admitted prior art indicates that chloroplast, mitochondria and nucleus transit peptides were well known and widely used at the time Applicant's invention was filed.

Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to utilize any of the known transit peptide sequences of the prior art, including the claimed chloroplast, mitochondria and nucleus transit peptides for their availability, in the transformation vector without any unexpected results. One skilled in the art would have been motivated to generate the claimed invention with a reasonable expectation of success.

Remarks

Claims 18, 26-27, 37, 49, 57, 46, 71-72, 79, and 100 are free of the prior art of record.

Papers related to this application may be submitted to Technology Sector 1 by facsimile transmission. Papers should be faxed to Crystal Mall 1, Art Unit 1638, using fax number (703) 308-4242. All Technology Sector 1 fax machines are available to receive transmission 24 hrs/day, 7 days/wk. Please note that the faxing of such papers must conform with the Notice published in the Official Gazette, 1096 OG 30 (November 15, 1989).

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Medina A. Ibrahim whose telephone number is (703) 306-5822. The Examiner can normally be reached Monday-Thursday from 8:30AM to 5:30PM and every other Friday 9:00AM to 5:00PM.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Dr. Amy Nelson, can be reached at (703) 306-3218.

Any inquiry of a general nature or relating to the status of this application should be directed to the receptionist whose telephone number is (703) 308-0196.

5/12/03

Mai

AMY J. NELSON, PH.D
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600